CLAIMS

1. A fusion polypeptide for expression in a host cell comprising a TolAIII domain or a functional homologue, fragment, or derivative thereof and a non-TolA polypeptide, wherein the TolAIII domain or functional homologue, fragment, or derivative thereof is located towards the N-terminus of the fusion polypeptide and the non-TolA polypeptide is located towards the C-terminus of the fusion polypeptide.

2. The fusion polypeptide according to claim 1, further comprising a signal peptide.

3. The fusion polypeptide according to claim 2, in which the signal peptide is located at or near the N-terminus of the fusion polypeptide.

4. The fusion polypeptide according to any preceding claim, wherein the TolAIII domain or functional homologue, fragment, or derivative thereof has been codon-optimised for expression in the host cell.

5. The fusion polypeptide according to any of the preceding claims, further comprising a linker between the TolAIII domain or functional homologue, fragment, or derivative thereof and the non-TolA polypeptide.

6. The fusion polypeptide according to claim 5, wherein the linker comprises at least one cleavage site for an endopeptidase.

7. The fusion polypeptide according to claim 6, wherein the cleavage site comprises the amino acid sequence DDDDK (SEQ ID NO: 3) and/or LVPR (SEQ ID NO: 4) and/or IEGR (SEQ ID NO: 5).

8. The fusion polypeptide according to any of the preceding claims, further comprising an affinity purification tag.

9. The fusion polypeptide according to claim 8, wherein the affinity purification tag is

located at or near the N-terminus of the fusion polypeptide.

10. The fusion polypeptide according to claim 9, wherein the affinity purification tag is an N-terminal His_n tag, with n=4, 5, 6, 7, 8, 9 or10 (SEQ ID NOs: 6 – 12, respectively; preferably n=6 [SEQ ID NO: 8]), optionally with the His_n tag linked to the fusion polypeptide by one or more Ser residues (preferably 2).

11. The fusion polypeptide according to any of the preceding claims, wherein the TolAIII domain consists of amino acid residues 329-421 (SEQ ID NO: 13) of the *Escherichia coli* TolA sequence (SwissProt Acc. No. P19934).

12. The fusion polypeptide according to any of the preceding claims, wherein the host cell is bacterial (for example, *Escherichia coli*).

13. The fusion polypeptide according to any of the preceding claims, wherein the non-TolA polypeptide is BCL-XL.

14. A DNA molecule encoding the fusion polypeptide as defined in any of claims 1-13.

15. A DNA molecule according to claim 14, wherein the mRNA properties of the DNA molecule when transcribed are optimised for expression in the host cell.

16. An expression vector comprising the DNA molecule according to either of claim 14 or claim 15 for expression of the fusion polypeptide defined in any of claims 1-13.

17. The expression vector according to claim 16, having an inducible promoter (for example, the IPTG-inducible T7 promotor) which drives expression of the fusion polypeptide.

18. The expression vector according to either of claim 16 or claim 17, having an antibiotic resistance marker (for example, the *bla* gene, which confers resistance to ampicillin and chloramphenicol).

19. A cloning vector for producing the expression vector defined in any of claims 16-18, comprising DNA encoding the TolAIII domain or a functional homologue, fragment, or derivative thereof upstream or downstream from a cloning site which allows in-frame insertion of DNA encoding a non-TolA polypeptide.

20. The cloning vector according to claim 19, further comprising DNA encoding at least one cleavage site (for example, the amino acid sequence DDDDK [SEQ ID NO: 3] and/or LVPR [SEQ ID NO: 4] and/or IEGR [SEQ ID NO: 5]) for an endopeptidase, the cleavage site located between the DNA encoding the TolAIII domain or a functional homologue, fragment, or derivative thereof and the cloning site.

21. The cloning vector according to either of claims 19 or 20, wherein the cloning site comprises at least one restriction endonuclease (for example, *Bam*HI and/or *Kpn*I) target sequence.

22. The cloning vector according to any of claims 19-21, further comprising DNA encoding an affinity purification tag as defined in either of claim 8 or claim 9.

23. The cloning vector according to any of claims 19-22, further comprising an inducible promoter (for example, the IPTG-inducible T7 promotor).

24. The cloning vector according to any of claims 19-23, further comprising DNA encoding an antibiotic resistance marker (for example, the *bla* gene, which confers resistance to ampicillin and chloramphenicol).

25. The cloning vector according to any of claims 19-24, having the structure of pTolE, pTolT or pTolX (as shown in Figure 2 with reference to the description).

26. Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of a fusion polypeptide as defined in any of claims 1-13.

27. Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of the DNA molecule as defined in either of claim 14 or claim 15.

28. Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of an expression vector as defined in any of claims 16-18.

29. Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of a cloning vector as defined in any of claims 19-25.

30. A host cell containing the DNA as defined in claim 13 and/or the expression vector as defined in any of claims 16-18 and/or the cloning vector as defined in any of claims 19-25.

31. Use of the fusion polypeptide as defined in any of claims 5-13 for immobilisation of the non-TolA polypeptide, comprising the step of:
binding the fusion polypeptide to a TolA binding polypeptide (e.g. the TolA-recognition site of colicin N or other colicins, the TolA binding region of bacteriophage g3p-D1 protein, or the TolA binding region of TolB or other Tol proteins).

32. Use of the fusion polypeptide as defined in any of claims 9-13 for immobilisation of the non-TolA polypeptide, comprising the step of:
binding the affinity tag of the fusion polypeptide to a binding moiety.

33. Use of the fusion polypeptide as defined in any of claims 5-13 for purification and isolation of the non-TolA polypeptide, comprising the steps of:
(i) binding the fusion polypeptide to a TolA binding polypeptide (e.g. the TolA-recognition site of colicin N or other colicins, the TolA binding region of bacteriophage g3p-D1 protein, or the TolA binding region of TolB or other Tol proteins);
(ii) cleaving the non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof using an endopeptidase; and
(iii) separating the cleaved non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof.

34. Use of the fusion polypeptide as defined in any of claims 8-13 for purification and isolation of the non-TolA polypeptide, comprising the steps of:
(i) binding the affinity tag of the fusion polypeptide to a binding moiety;
(ii) cleaving the non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof using an endopeptidase; and
(iii) separating the cleaved non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof.

35. Use of the fusion polypeptide as defined in any of claims 1-13 for studying interaction properties of the non-TolA polypeptide or the fusion polypeptide, for example self-interaction, interaction with another molecule, or interaction with a physical stimulus.

36. A method for high expression of a polypeptide as a fusion polypeptide in a host cell, comprising the step of expressing the polypeptide as a fusion polypeptide as defined in any of claims 1-13 in a host cell.

The present invention relates to fusion proteins (fusion polypeptides), particularly for use in expression and/or purification systems. The present inventors have found that the To1AIII domain has remarkable properties which are of particular use as a fusion protein partner to achieve high levels of expression in a host cell. In one aspect of the invention, a To1AIII domain or a functional homologue, fragment, or derivative thereof is located towards the N-terminus of the fusion polypeptide and a non-To1A polypeptide is located towards the C-terminus of the fusion polypeptide.